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Romanian Prader-Willi Association

ASOCIACIÓN MADRILEÑA  
PARA EL SÍNDROME DE  
PRADER-WILLI



## DNA EXTRACTION FROM DRIED BLOOD SPOTS AND METHYLATION TEST FOR DIAGNOSIS OF PRADER-WILLI SYNDROME PATIENTS

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Prader-Willi syndrome (PWS) is a complex genetic disease that arises from lack of expression of paternally inherited imprinting controlled genes on chromosome 15q11-q13. The frequency is between 1/10000 and 1/30000. An early molecular diagnosis of the syndrome can help not only the correct treatment of the condition but can help the family to begin an optimal lifestyle for the PWS patients since an early age.

The B.I.R.D. Foundation developed a cost effective diagnostic procedure for PWS starting from dried blood spots on filter paper. In particular, a methylation test in the Prader-Willi chromosome region (PWCR) is performed. It consists of a chemical modification with sodium bisulphite which involves the addition of a methyl group to the carbon-5 of the cytosine pyrimidine ring. Cytosine converts to uracil, except when it is methylated because 5-methylcytosine is resistant to sodium bisulphite. In a normal person, sodium bisulphite modifies the paternal genes of PWCR and not the maternal genes that are methylated. In PWS patients only methylated DNA is present in the PWCR either because there is a deletion in the paternal DNA or there is UPD or even other rearrangements or imprinting defects. Subsequently we amplified the sodium bisulphite treated PWCR with a methylation-specific PCR with two pairs of primers that give amplicons of different sizes. By gel electrophoresis it is possible to see two bands if there are both the methylated and non methylated fragment and a single band if only the methylated band is present, permitting us to diagnose the PWS with a sensitivity of 99%.

The B.I.R.D. Foundation, in collaboration with the IPWSO, is today able to offer the diagnosis for PWS free of charge to anyone who requires it. The test is performed on DNA extracted from dried blood spots that represent an easy and low cost way to transport samples. The test performed allows a quick diagnosis that takes not more than a week. Fast results are essential in order to begin the therapy with growth hormone (GH) as soon as possible since the treatment can radically change the clinical phenotype of PWS.